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BAYER AG TOXICOLOGY LAB

Document Title
SALMONELLA/MICROSOME PLATE TEST ON BENZENE, 1,1'-METHYLENEBIS(ISOCYANATO- WITH TSCA HEALTH & SAFETY STUDY COVER SHEET DATED 02/10/1997

Chemical Category
BENZENE, 1,1'-METHYLENEBIS(ISOCYANATO- (26447-40-5)

OFFICE OF TOXIC SUBSTANCES

REV. 7/27/82

CODING FORM FOR GLOBAL INDEXING

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TSCA HEALTH & SAFETY STUDY COVER SHEET

TSCA CBI STATUS:

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1.0 SUBMISSION TYPE ☐*Contains CBI*

X 8(d) ☐ 8(e) ☐ FYI ☐ 4 ☐ OTHER: Specify_____

X - Intial Submission ☐ Follow-up Submission X =Final Report Submission

Previous EPA Submission Number or Title if update or follow-up: Docket Number, if any: #

☐ continuation sheet attached

2.1 SUMMARY/ABSTRACT ATTACHED (may be required for 8(e): optional for §4, 8(d) & FYI) ☐ YES **X** NO	**2.2 SUBMITTER TRACKING NUMBER OR INTERNAL ID** Cert# P 917006659 97-12-3	**2.3 FOR EPA USE ONLY**

3.0 CHEMICAL/TEST SUBSTANCE IDENTITY ☐*Contains CBI*

Reported Chemical Name (specify nomenclature if other than CAS name):

CAS#: 26447-40-5 Benzene, 1,1'-methylenebis(isocyanato-

Purity ____%

☐ Single Ingredient

☐ Commerical/Tech Grade

-Mixture *Trade Name:* VP PU 1806 *Common Name:*

Contains No CB

CAS Number	*NAME*	*% WEIGHT*

Other chemical(s) present in tested mixture

☐ continuation sheet attached

4.0 REPORT/STUDY TITLE ☐*Contains CBI*

SALMONELLA/MICROSOME TEST

☐

86970000704

5.1 STUDY/TSCATS INDEXING TERMS

[CHECK ONE]

HEALTH EFFECTS (HE):_____ ENVIRONMENTAL EFFECTS (EE):_____ ENVIRONMENTAL FATE (EF):_____

5.2 STUDY/TSCATS INDEXING TERMS (see instructions for 4 digit codes)

STUDY TYPE: _____	SUBJECT ORGANISM (HE, EE only):_____	ROUTE OF EXPOSURE (HE only):_____	VEHICLE OF EXPRE (HE)_____
Other:	*Other:*	*Other:*	*Other:*

6.0 REPORT/STUDY INFORMATION ☐*Contains CBI* - Study is GLP ____

Laboratory Bayer AG Tox Lab Report/Study Date 11/12/96

Source of Data/Study Sponsor (if different than submitter) Bayer Number of pages : 48

☐ continuation sheet attached

7.0 SUBMITTER INFORMATION ☐*Contains CBI*

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P[illegible]**burgh, PA 15205-9741** Submitter Address (if different): _____

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☐ continuation sheet attached

8.0 ADDITIONAL/OPTIONAL STUDY COMMENTS ☐*Contains CBI*

☐ continuation sheet attached

Submitter Signature: F J Rattay **Date:** 2/10/97

Page 1 of 1

cc: D. W. Lamb

B A Y E R A G
TOXICOLOGY
Friedrich-Ebert-Straße 217-333
D-42096 Wuppertal, F.R.G.

Report No. : 25625
Report Date: 12.11.1996

VP PU 1806
(in DMSO)

SALMONELLA/MICROSOME TEST

Contains No CBI

Study No.: T 5053818

by

Dr. B. Herbold

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GLP Compliance Statement

Compound : VP PU 1806
Study No. : T 5053818

The study was not conducted in compliance with the OECD Principles of Good Laboratory Practice (GLP) and with the principles of Good Laboratory Practice (GLP) according to Annex 1 ChemG (Bundesanzeiger Nr. 42a of the 2nd of March 1983 and Bundesgesetzblatt, Part I, of the 29th of July 1994).

The deviations were as follows:

No data were available on analytical investigations of the compound and on its stability in solution. The same was true for storage stability.

No inspections were performed by the quality assurance unit.

These deviations did not limit the assessment of the results.

Dr. B. Herbold

Wuppertal, November 6, 1996

C O N T E N T S

1. Signatures

Study Director : Dr. B. Herbold — November 6, 1996 (Date)

Section Head : Dr. E. Bomhard — Nov. 11, 1996 (Date)

2. Summary

VP PU 1806 was investigated using the Salmonella/microsome test for point mutagenic effects in doses of up to 5000 µg per plate on four Salmonella typhimurium LT2 mutants. These comprised the histidine-auxotrophic strains TA 1535, TA 100, TA 1537 and TA 98.

Doses up to and including 50 µg per plate did not cause any bacteriotoxic effects: Total bacteria counts remained unchanged and no inhibition of growth was observed. At higher doses, the substance had a strong, strain-specific bacteriotoxic effect, so that this range could only be used to a limited extent up to 900 µg per plate for assessment purposes. Substance precipitation occurred at the dose 5000 µg per plate.

Evidence of mutagenic activity of VP PU 1806 was seen. On Salmonella typhimurium TA 100 and TA 98, a biologically relevant increase was found in the mutant count compared to the corresponding negative control. Positive response was found only with S9 mix containing 30% S9 fraction. The lowest effective dose was 75 µg per plate. The Salmonella/microsome test thus showed VP PU 1806 to have a mutagenic effect.

The positive controls sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine and 2-aminoanthracene had a marked mutagenic effect, as was seen by a biologically relevant increase in mutant colonies compared to the corresponding negative controls.

3. Introduction

The mutagenicity evaluation was performed using the Salmonella/microsome test, also termed the Ames Test, as described by Ames et al. (1973a, 1975) and Maron and Ames (1983).

The Salmonella/microsome test is a screening method which detects point mutation caused by chemical agents in vitro. Auxotrophic mutants of Salmonella typhimurium are used to demonstrate this effect. For this purpose, the rate of reversion to prototrophy is evaluated in negative control and treated groups. A mutagenic effect is assumed if this rate increases sufficiently in the treated groups.

Mammalian metabolism, which is of great significance in chemical mutagenesis, is simulated in this test by the 9000 g fraction of homogenized mammalian livers. Together with cofactors, this forms the "S9 mix" which represents the metabolic model in this test.

The method itself is considered to be very sensitive (Herbold et al., 1976; Herbold, 1978) and is well suited for fast screening. Available literature indicates a high correlation between the positive and negative responses of the Ames assay and the carcinogenic activity of the tested substances (McCann et al., 1975a, 1976; Purchase et al., 1976, 1978). In addition, the test represents a good screening system for potential carcinogenic effects, although the results should not be overrated, as this high correlation may not apply to all substance groups (Ames, 1979; Andrews et al., 1978; Clayson, 1980; Glatt et al., 1979 and Rinkus and Legator, 1979; Zeiger, 1987).

The test was performed at the Carcinogenicity and Genotoxicity Unit of Toxicology, BAYER AG, Friedrich-Ebert-Straße 217-333, D-42096 Wuppertal, F.R.G.

Study initiation date:	September 9, 1996
Study start date:	October 2, 1996
Study termination date:	November 4, 1996
Study completion date:	report date (see front page)

The records are filed in the Toxicology's archive.

4. Material and Methods

4.1 Substances

4.1.1 Test Substance

name of test substance	:	VP PU 1806
manufacturer	:	BAYER AG
batch number	:	not indicated by the sponsor
content	:	not indicated by the sponsor
approved	:	not indicated by the sponsor
appearance	:	solid, humid light-beige mass
storage	:	refrigerator
intended use	:	industrial chemical

4.1.2 Positive Controls

Sodium azide (Na azide, SERVA), order no. 30175 (Control:D), a direct-acting mutagen used as specific positive control for TA 1535.

Nitrofurantoin (NF, SIGMA), order no. N-7878, lot no. 67F0765, a direct-acting mutagen used as specific positive control for TA 100.

4-nitro-1,2-phenylene diamine (4-NPDA, Fluka), order no. 73630, analysis no. 247722/1 194, a direct-acting mutagen used as specific positive control for TA 1537 and TA 98.

2-aminoanthracene (2-AA, Aldrich), order no. A3,880-0, batch no. 52234-024, a promutagen which reverts all the strains and serves as a control for the activating effect of the S9 mix.

The positive controls sodium azide, nitrofurantoin and 4-nitro-1,2-phenylene diamine were only used without S9 mix; the positive control 2-aminoanthracene was only used with S9 mix.

4.2 Indicator Organisms

4.2.1 Description of Test Strains

Histidine-deficient mutants of Salmonella typhimurium LT2 served as indicators to demonstrate point mutagenic effects. These strains were selected specifically for the Salmonella/microsome test. Since point mutations can be divided into two basic classes, base-pair substitutions and frameshift mutations, several strains were used which cover both types.

These included the strains selected by Ames et al. (1973b), Salmonella typhimurium TA 1535 and TA 1537, as well as Salmonella typhimurium TA 100 and TA 98, developed by McCann et al. (1975b). TA 1535 and TA 100 bear the base-pair substitution, his G 46, and TA 100 additionally contains the plasmid pKM 101. This R factor, also contained in TA 98, codes for an ampicillin resistance and should raise the sensitivity of both strains. TA 1537 and TA 98 bear frameshift markers. TA 1537 exhibits the +1 mutant, his C 3076, while TA 98 bears the +2 type, his D 3052.

Furthermore, all the strains have two other properties in common, which should increase their sensitivity. Firstly, they are deep rough, i.e. partly deficient in lipopolysaccharide side chains in their cell walls, enabling larger molecules to penetrate the bacterial cell wall and produce mutations. Secondly, their reduced UV repairability partly prevents the repair of damage, such as that triggered off by UV light (e.g. thymine dimers), and thus gives rise to mutations.

Whereas TA 1538 was used in addition to TA 100, TA 1538 is not normally used in addition to TA 98. This has two reasons:

a) There is no relevant increase in the spontaneous mutant counts of TA 98, compared to the spontaneous range of TA 1538. Special differences in sensitivity existing between TA 1535 and TA 100, and attributed to the relatively high spontaneous rate of TA 100 (10 times that of TA 1535), do not exist between TA 1538 and TA 98. b) An international general inquiry had shown, that using TA 1538 in addition to any of the test strains in this study would not provide further information of biological relevance (Herbold, 1983).

This is in agreement with international guidelines, as published by OECD, EEC, or EPA. Strain TA 1538 was either deleted in these guidelines, or never introduced at all. Maron and Ames (1983) also reported: "Although TA 1538 is useful for the detection of particular aromatic frameshift mutagens such as 4-nitro-o-phenylene diamine, we decided to drop the strain because it overlaps considerably with TA 98".

TA 1538, which differs from TA 98 in lacking the plasmid pKM 101, is used in spite of these considerations, if questionable TA 98-results need clarification. This was not the case in the present investigation, however.

4.2.2 Origin of Strains

The original strains were obtained from Prof. Bruce Ames and arrived at Fachbereich Toxicology, BAYER AG, on February 9, 1993.

4.2.3 Production of Stock Cultures

Immediately upon receipt, the samples were inoculated on nutrient agar plates, to which ampicillin had been added for the TA 100 and TA 98 strains. These plates were incubated at 37°C for approximately 24 hours. Samples were taken from individual colonies with a sterile inoculation loop, and transferred to nutrient broth. In the case of TA 100 and TA 98, ampicillin had also been added to this broth. The samples were again incubated overnight at 37°C. New samples of these cultures were inoculated onto nutrient agar plates, which had again been provided with ampicillin for TA 100 and TA 98.

After an incubation period of approximately 24 hours at 37°C, new samples of individual colonies from these plates were transferred to flasks containing approximately 20 ml normal nutrient broth. This inoculum was incubated overnight at 37°C, after which a small sample was taken to check the genotype. At the same time, the remaining cultures were treated with DMSO to protect against the effects of freezing, and immediately frozen at -80°C in 1 ml portions (Ames et al., 1973b; McCann et al., 1975b). No ampicillin-resistance test was done on the samples used for testing genotype since the cultures had already been sufficiently selected by the ampicillin.

In addition to the test for crystal-violet sensitivity (deep rough character), a test was done for UV sensitivity (uvrB). The crystal-violet and UV sensitivity tests are described below. The frozen cultures which did not produce satisfactory results here were discarded. Remaining cultures were stored for future tests. In addition, frozen cultures of batches with unsatisfactory negative and/or positive control results in the definitive tests were also discarded.

Whenever new stock cultures needed to be produced, individual cultures grown on nutrient agar were used, to which ampicillin had been added for the TA 100 and TA 98 strains. Samples of these individual colonies were then transferred to approximately 20 ml nutrient broth, incubated, divided up, and checked for crystal-violet and UV sensitivity.

One 1 ml-portion was thawed for each test and strain, and quantities of 0.2 ml of the thawed culture were added to 10 ml nutrient broth. This culture was incubated overnight at 37°C and used only on the same day. A new, small stock culture, which had been checked for its properties directly before freezing, was thus available for each individual test. In general this obviated any need to re-check the genotype for each Salmonella/microsome test. This procedure is in accordance with the methods described by Ames et al. (1975) and Maron and Ames (1983).

4.2.4 Checking of Genotype

4.2.4.1 Histidine Requirement

In each individual test, histidine dependence of the cultures was automatically checked by the accompanying negative controls. The number of mutants per individual plate is listed in the Tables 1 to 12.

4.2.4.2 Ampicillin Resistance (pKM 101)

A special test for ampicillin resistance was not necessary since strains TA 100 and TA 98 were incubated on ampicillin containing nutrient agar and formed individual colonies. Consequently surviving bacteria were ampicillin resistant.

4.2.4.3 Crystal-Violet Sensitivity (deep rough)

A quantity of 0.1 ml was taken from the samples of individual stocks and spread onto nutrient agar, using four plates per strain. After a few minutes, filter papers, to which 10 µl of an aqueous, crystal-violet solution had been added at a concentration of 1 mg/ml, were placed in the middle of the plates. The plates were then incubated overnight at 37°C. The diameters of the inhibition zones that had formed were then measured. The inhibition zones of all stock batches used indicated an adequate sensitivity to crystal-violet.

4.2.4.4 UV Sensitivity (uvrB)

As described under 4.2.4.3, samples were spread onto nutrient agar plates. One half of each plate was covered with aluminium foil and irradiated without a lid for six seconds (TA 1535 and TA 1537) or eight seconds (TA 100 and TA 98) with UV light of a wavelength of 254 nm at a distance of 33 cm. The irradiated plates were incubated as described under 4.2.4.3 and checked. To demonstrate adequate sensitivity in this test, cultures had to show an inhibition of growth over half their area, i.e. no bacteria should have grown on the irradiated half. This was the case with all the stock batches used.

4.2.5 Stock Batches

Stock Batches Used in Tables 1-10	11-12	Strain
09.08.96/1		TA 1535
09.08.96/1	09.08.96/2	TA 100
09.08.96/1		TA 1537
09.08.96/1	09.08.96/2	TA 98

4.3 S9 Mix

S9 mix was used to simulate the mammalian metabolism of the test substance. It was made from the livers of at least six adult male Sprague Dawley rats, of approximately 200 to 300 g in weight. For enzyme induction, the animals received a single intraperitoneal injection of Aroclor 1254, dissolved in corn oil, at a dose of 500 mg/kg body weight, five days prior to sacrifice. The animals were prepared unfasted, following the directions of Ames et al. (1975) and Maron and Ames (1983).

The rats were terminated. Livers were removed under sterile conditions immediately after sacrifice and kept at 4°C until all animals had been prepared. All the remaining steps were carried out under sterile conditions at 4°C.

The livers were washed with cold (4°C), 0.15 M KCl solution (approximately 1 ml KCl per 1 g liver), and then homogenized in fresh, cold (4°C), 0.15 M KCl (approximately 3 ml KCl per 1 g liver). The homogenate was then centrifuged in a cooling centrifuge at 4°C and 9000 g for 10 minutes. The supernatant (the S9 fraction) was stored at -80°C in small portions.

These portions were slowly thawed before use. The S9 mix was freshly prepared (Ames et al., 1973a) and used only on the same day. It was placed in a vessel with a double glass wall until used. The hollow wall was filled with ice to keep the S9 mix cold.

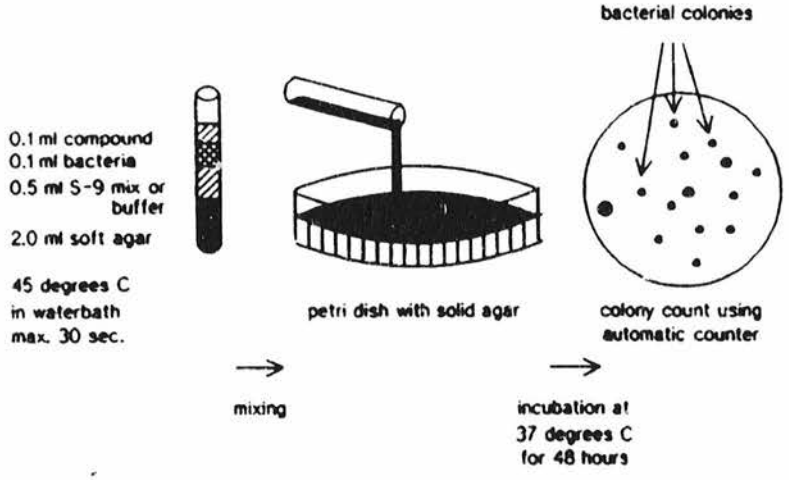
Seventy ml of cofactor solution are composed as follows:

$MgCl_2 \times 6 H_2O$	162.6 mg
KCl	246.0 mg
glucose-6-phosphate, disodium salt	179.1 mg
NADP, disodium salt	315.0 mg
phosphate buffer	100.0 mM

S9 mix consists of this cofactor solution, S9 fraction and, if needed, 0.15 M KCl. The amount of S9 fraction in S9 mix is indicated in Tables 1 to 12 in percent. The S9 mix comprised the amount of S9 fraction (x%) indicated in Tables 1 to 12, 70% cofactor solution and (30-x)% 0.15 M KCl. The S9 fraction was derived from the preparation dated February 13, 1996 (protein content 28.4 mg per ml). Prior to first use, each batch was checked for its metabolizing capacity by using reference mutagen(s); appropriate activity was demonstrated. At the beginning of each experiment 4 aliquots of the S9 mix were plated (0.5 ml per plate) in order to assess its sterility. This was repeated after completion of test tube plating. The sterility control plates were then incubated for 48 hours at 37°C. No indication of contamination of S9 mix was found.

4.4 Test Design

The test followed the directions of Ames et al. (1973a, 1975) and Maron and Ames (1983).



For the mutant count, four plates were used, both with and without S9 mix, for each strain and dose. An equal number of plates, filled with the solvent minus the test substance, comprised the negative control. Each positive control also contained four plates per strain. The amount of solvent for the test substance and for the controls was 0.1 ml/plate.

The doses for the first trial were routinely determined on the basis of a standard protocol: if not limited by solubility 5000 µg or 5 µl per plate were used as the highest dose. At least five additional doses were routinely used. If less than three doses were used for assessment, at least two repeats were performed. The results of the first experiment were then considered as a pre-test for toxicity. However, in case of a positive response or if at least three doses could be used for assessment, the first trial was included in the assessment. If the second test confirmed the results of the first, no additional repeat was performed. Doses of repeats were chosen on the basis of the results obtained in the first experiment.

The toxicity of the substance was assessed in three ways. The first method was a gross appraisal of background growth on the plates for mutant determination. If a reduction in background growth was observed, it was indicated in the tables by the letter "**b**" after the mutant count. Where only a single "**b**", without any other values, is noted for a concentration, this "**b**" represents four plates with reduced background growth. (The same applies to the signs "**c**", "**v**", "**p**", "**n**" or "**%**", which may also be used in the tables.) Secondly, a toxic effect of the substance was assumed when there was a marked and dose-dependent reduction in the mutant count per plate, compared to the negative controls. Thirdly, the titer was determined. Total bacterial counts were taken on two plates for each concentration studied with S9 mix. However, if an evaluation was performed only without S9 mix, the bacterial count was taken without S9 mix.

The bacterial suspensions were obtained from 17-hour cultures in nutrient broth, which had been incubated at 37°C and 90 rpm. These suspensions were used for the determination of mutant counts. No standardized procedure was employed to set the bacterial suspensions at a defined density of viable cells per milliliter, since the chosen method of incubation normally produces the desired density. However, the numbers of viable cells were established in a parallel procedure by determining the titers. The results of these determinations may be seen in the negative controls in Tables 1 to 12.

The dilution of bacterial suspensions used for the determination of titers was 1:1,000,000. Titers were determined under the same conditions as were the mutations, except that the histidine concentration in the soft agar was increased five-fold to permit the complete growth of bacteria.

The tests were performed both with and without S9 mix. Full details are given in the Tables 1 to 12.

The count was made after the plates had been incubated for 48 hours at 37°C. If no immediate count was possible, plates were temporarily stored in a refrigerator.

The following criteria determined the acceptance of an assay:
a) The negative controls had to be within the expected range, as defined by published data (e.g. Maron and Ames, 1983) and/or the laboratories' own historical data (see Chapter 8).
b) The positive controls had to show sufficient effects, as defined by the laboratories' experience (see Chapter 8).
c) Titer determinations had to demonstrate sufficient bacterial density in the suspension.

Only trials which complied with all three of the above criteria were accepted for assessment. Even if the criteria for points (b) and (c) were not met, a trial was accepted if it showed mutagenic activity of the test compound. Furthermore, an unacceptable trial would have been repeated.

The following doses per plate were evaluated in the first test:

		µg per plate	
1.	Negative control	0	
2.	VP PU 1806	5000	
3.	VP PU 1806	1581	
4.	VP PU 1806	500	
5.	VP PU 1806	158	
6.	VP PU 1806	50	
7.	VP PU 1806	16	
8.	Positive control, sodium azide	10	(only TA 1535)
9.	Positive control, nitrofurantoin	0.2	(only TA 100)
10.	Positive control, 4-nitro-1,2-phenylene diamine	10	(only TA 1537)
11.	Positive control, 4-nitro-1,2-phenylene diamine	0.5	(only TA 98)
12.	Positive control, 2-aminoanthracene	3	

Due to the substance's toxicity, doses ranging from 19 µg to 900 µg per plate were chosen for the repeat tests. Individual doses are given in Tables 5 to 12.

VP PU 1806 was dissolved in DMSO (dryed with a molecular sieve, 0.4 nm) and formed a clear colorless solution. The positive controls were dissolved in DMSO.

The solvent used was chosen out of the following solvents, in the order given: water, DMSO, methanol, ethanol, acetone, ethylene glycol dimethylether, and DMF according to information given by the internal sponsor. The order of these solvents is based on their bacteriotoxic effects in preincubation experiments.

No "untreated" negative control was set up for DMSO, since sufficient evidence was available in the literature (e.g. Maron and Ames, 1983) and from our own experience (see Chapter 8), indicating that this solvent had no influence on the spontaneous mutant counts of the bacterial strains used.

4.5 Assessment Criteria

A reproducible and dose-related increase in mutant counts of at least one strain is considered to be a positive result. For TA 1535, TA 100 and TA 98 this increase should be about twice that of negative controls, whereas for TA 1537, at least a threefold increase should be reached. Otherwise, the result is evaluated as negative. However, these guidelines may be overruled by good scientific judgement.

In case of questionable results, investigations should continue, possibly with modifications, until a final evaluation is possible.

4.6 Study Guidelines

The study was performed according at least to the following guidelines:

EEC Directive 92/69/EEC
B.14. Salmonella typhimurium
Reverse Mutation Test

OECD Guidelines for Testing of Chemicals
"Genetic Toxicology: Salmonella typhimurium,
Reverse Mutation Assay"
Adopted: 26 May 83, No. 471

Health Effects Test Guidelines, June 1996. (U.S.)
Environmental Protection Agency Washington, DC, (EPA712-C-96-219)
OPPTS 870.5265 - The Salmonella typhimurium
Reverse Mutation Assay - "Public Draft"

4.7 Study Identification and Responsibilities

4.7.1 Type of Test and Study Number

Salmonella/Microsome Test :T 5053818

4.7.2 Responsibilities

Head of Toxicology	:Prof. Dr. G. Schlüter
Senior Expert Genotoxicity	:Dr. B. Herbold
Study Director	:Dr. B. Herbold
Senior Technician	:Mrs. M. Bönning
Head of Archives	:Prof. Dr. G. Schlüter

5. Results

5.1 Description of Results

The colony number of each plate and mean values are listed for each dose in Tables 1 to 12. As may be seen, there was no indication of a bacteriotoxic effect of VP PU 1806 at doses of up to and including 50 µg per plate. The total bacteria counts consistently produced results comparable to the negative controls, or differed only insignificantly. No inhibition of growth was noted as well. Higher doses had a strong, strain-specific bacteriotoxic effect. Therefore they could only partly be used for assessment purposes up to and including 900 µg per plate. At 5000 µg per plate, the substance precipitated.

None of the four strains concerned showed a dose-related and biologically relevant increase in mutant counts over those of the negative controls. This applied both to the tests with and without S9 mix (Tables 1 to 4) and was confirmed by the results of the repeat tests (Tables 5 to 12). However, with S9 mix, which contained 10% S9 fraction, increases were seen for TA 100 and TA 98 (Tables 2, 4, 6, 8, 9 and 10), which reached, however, never a biologically relevant level.

Therefore an additional repeat was performed with TA 100 and TA 98 using an S9 mix, which contained 30% S9 fraction. Both strains revealed a dose-related increase in mutant counts to well over double those of negative controls (Tables 11 and 12). The lowest active dose was 75 µg per plate.

Summary of the Results with VP PU 1806
in the Salmonella/Microsome Test

S9 mix		TA 1535	TA 100	TA 1537	TA 98
without		-ve	-ve	-ve	-ve
with	10%	-ve	-ve	-ve	-ve
	30%	-ve	+ve	-ve	+ve

-ve = negative, +ve = positive

The positive controls sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine and 2-aminoanthracene increased mutant counts to well over those of the negative controls, and thus demonstrated the system's sensitivity and the activity of the S9 mix.

5.2 Tabulated Summary of Data

Summary of Mean Values Without S9 Mix

Table and group µg/plate	Strain TA 1535	TA 100	TA 1537	TA 98
1-4				
0	6	79	8	21
16	9	70	9	29
50	10	65	5	24
158	8	62	5	26
500	8	56	4	16
1581	1	0	0	0
5000	--	--	-	--
Na-azide	933			
NF		242		
4-NPDA			171	181
5-8				
0	8	84	8	21
19	9	86	6	25
38	10	76	7	24
76	10	78	6	21
152	10	86	10	22
304	10	78	7	17
608	--	29	--	7
Na-azide	838			
NF		228		
4-NPDA			92	173

Summary of Mean Values With S9 Mix From

Table and group µg/plate	Strain TA 1535	TA 100	TA 1537	TA 98
1-4				
0	9	92	6	28
16	7	124	8	33
50	11	124	9	45
158	8	122	5	33
500	11	104	7	45
1581	7	2	0	0
5000	--	---	-	--
2-AA	189	1147	293	1916
5-8				
0	12	89	6	26
19	9	130	9	34
38	7	153	8	48
76	11	141	7	48
152	8	147	7	39
304	9	137	8	50
608	--	42	4	22
2-AA	235	903	256	1728

Table and group µg/plate	Strain TA 100		TA 98	
	10%	30%	10%	30%
9-12				
0	91	105	27	32
75	132	304	45	103
150	122	353	37	71
300	142	291	38	73
450	142	118	38	39
600	80	133	35	47
750	71	86	28	32
900	63	100	24	39
2-AA	605	359	1587	653

6. Assessment

The Salmonella/microsome test, employing doses of up to 5000 µg per plate, showed VP PU 1806 to produce bacteriotoxic effects at 158 µg per plate and above. Therefore, 1581 µg per plate and above could not be used for assessment. Substance precipitation occurred at 5000 µg per plate.

Evaluation of individual dose groups, with respect to relevant assessment parameters (dose effect, reproducibility), revealed with S9 mix containing 30% S9 fraction biologically relevant variations from the respective negative controls for TA 100 and TA 98. These were regarded as mutagenic effects of VP PU 1806. The Salmonella/microsome test showed VP PU 1806 to be a mutagen.

In spite of the low doses used, positive controls increased the mutant counts to well over those of the negative controls, and thus demonstrated the system's high sensitivity.

Due to this sensitivity, indications of mutagenic effects of VP PU 1806 could be found at assessable doses of up to 900 µg per plate in Salmonella typhimurium TA 100 and TA 98.

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3. Historical Controls

Summary of historical negative and positive controls of experiments performed from January to June 1991 using mean values presented as medians (Z) and semi-Q range (QR)

Compound and S9 Mix	Strain TA 1535 Z	TA 1535 QR	TA 100 Z	TA 100 QR	TA 1537 Z	TA 1537 QR	TA 98 Z	TA 98 QR
water -	12	3	111	10	9	2	28	5
DMSO -	13	2	113	14	10	2	30	3
DMF -	9	-	80	--	7	-	23	-
methanol-	11	2	105	14	8	2	29	5
ethanol -	12	1	96	15	9	2	31	5
acetone -	10	-	55	--	5	-	21	-
EGDE[2] -	11	3	108	5	8	1	23	8
Na-azide-	623	102						
NF -			398	56				
4-NPDA -					49	10	89	20
30%								
water +	16	3	152	15	12	2	38	7
DMSO +	18	3	154	11	12	2	40	7
DMF +	11	-	84	--	9	-	29	-
methanol+	23	5	152	7	10	3	48	10
ethanol +	19	3	127	17	10	3	43	6
acetone +	14	-	84	--	14	-	18	-
EGDE[2] +	15	4	132	6	8	1	40	9
2-AA +	182	33	800	163	86	24	472	105
10%								
water +	15	-	102	--	5	-	46	-
DMSO +	16	3	132	5	10	1	39	4
methanol+	--	-	150	--	--	-	--	-
2-AA +	208	48	1408	216	314	14	754	369

[2]) Ethylene glycol dimethylether

Summary of historical negative and positive controls of experiments performed from July to December 1991 using mean values presented as medians (Z) and semi-Q range (QR)

Compound and S9 Mix	Strain TA 1535 Z	QR	TA 100 Z	QR	TA 1537 Z	QR	TA 98 Z	QR
water -	12	3	89	10	9	3	27	4
buffer -	13	2	97	10	8	1	25	2
DMSO -	12	3	92	15	9	1	24	4
DMF -	7		75		7		17	
methanol-	10	1	84	11	8	1	25	3
ethanol -	12	4	80	8	8	3	23	4
acetone -	12	2	87	6	8	1	26	4
EGDE[2] -	14	3	107	22	8	1	26	5
Na-azide-	605	122						
NF -			339	52				
4-NPDA -					53	9	79	17
30%								
water +	19	4	138	21	13	2	33	4
buffer +	17		159		13		38	
DMSO +	19	3	130	11	10	2	33	4
DMF +	11		142		9		32	
methanol+	25		134		12		37	
ethanol +	18	5	119	19	11	2	37	2
acetone +	18	2	111	9	13		28	11
EGDE[2] +	22	4	144	11	13	3	32	3
2-AA +	164	38	727	139	91	32	520	161
10%								
water +	16	4	113	18	10	3	33	5
buffer +	14		94		10		34	
DMSO +	16	2	118	14	10	3	31	3
DMF +	15		114	6	11		21	
methanol+	16		111		9		29	
ethanol +	19	3	94	6	12	2	32	2
acetone +	17		112		11		32	
EGDE[2] +	20	2	153	11	11	1	34	5
2-AA +	197	50	1431	260	304	116	1097	207

[2]) Ethylene glycol dimethylether

Summary of historical negative and positive controls of experiments performed from January to June 1992 using mean values presented as medians (Z) and semi-Q range (QR)

Compound and S9 Mix		Strain TA 1535 Z	TA 1535 QR	TA 100 Z	TA 100 QR	TA 1537 Z	TA 1537 QR	TA 98 Z	TA 98 QR
water	-	16	2	93	11	9	2	23	4
DMSO	-	15	4	81	9	9	1	23	4
DMF	-	13	3	68	4	7	1	19	3
ethanol	-	15	4	91	15	7	2	25	2
acetone	-	13	2	59	12	7	1	19	1
EGDE[2]	-	17		69		7		14	
Na-azid	-	660	147						
NF	-			285	65				
4-NPDA	-					52	7	74	13
30%									
water	+	23	4	124	18	12	1	31	6
DMSO	+	22	5	120	20	12	2	31	5
DMF	+	18	2	89	5	11	1	27	1
ethanol	+	25		145		9		38	
acetone	+	17	3	79	9	8	2	24	4
EGDE[2]	+	19		119		8		18	
2-AA	+	151	17	669	208	62	13	382	111
10%									
water	+	20	4	118	17	12	3	33	5
DMSO	+	17	3	111	15	10	2	32	4
DMF	+	17		87		9		34	
ethanol	+	24	4	98	5	9	2	37	5
acetone	+	15	3	69	7	12	6	29	5
EGDE[2]	+	11		48		11		22	
2-AA	+	159	35	1148	332	246	21	1126	292

[2]) Ethylene glycol dimethylether

Summary of historical negative and positive controls of experiments performed from July to December 1992 using mean values presented as medians (Z) and semi-Q range (QR)

Compound and S9 Mix	Strain TA 1535 Z	QR	TA 100 Z	QR	TA 1537 Z	QR	TA 98 Z	QR
water -	13	2	66	8	11	2	28	4
DMSO -	14	2	61	7	10	1	26	5
methanol-	11	-	64	-	7	-	30	-
ethanol -	11	1	57	4	8	2	27	5
acetone -	12	-	60	-	10	-	20	-
EGDE[2] -	13	1	54	13	8	1	23	2
Na-azid -	729	108						
NF -			251	27				
4-NPDA -					52	5	84	16
30%								
water +	21	-	108	-	12	-	34	-
DMSO +	18	1	96	13	11	1	30	2
ethanol +	25	-	84	-	12	-	47	-
acetone +	19	-	86	-	15	-	23	-
EGDE[2] +	22	-	68	-	11	-	30	-
2-AA +	152	45	491	114	80	16	538	34
10%								
water +	19	3	79	16	12	2	38	6
DMSO +	18	5	85	12	13	3	38	6
methanol+	20	-	78	-	10	-	42	-
ethanol +	19	-	93	-	13	-	40	-
acetone +	19	-	83	-	11	-	33	-
EGDE[2] -	14	4	71	20	10	5	34	2
2-AA +	179	65	978	220	254	53	1129	226

[2]) Ethylene glycol dimethylether

Summary of historical negative and positive controls of experiments performed from January to June 1993 using mean values present medians (Z) and semi-Q range (QR)

Compound and S9 Mix		Strain TA 1535 Z	QR	TA 100 Z	QR	TA 1537 Z	QR	TA 98 Z	QR
water	-	13	2	96	13	10	3	25	4
DMSO	-	11	2	80	9	8	1	22	3
DMF	-	10	-	70	-	11	-	16	-
ethanol	-	10	1	77	-	10	-	26	-
acetone	-	14	1	88	11	9	1	25	3
EGDE[2]	-	14	2	95	14				
Na-azid	-	683	86						
NF	-			337	52				
4-NPDA	-					57	9	82	15
30%									
water	+	17	-	116	-	11	-	36	-
DMSO	+	15	2	111	7	9	2	30	5
ethanol	+	28	-	135	-	10	-	38	-
EGDE[2]	+	36	-	137	-	14	-	45	-
2-AA	+	157	35	571	190	82	25	494	76
10%									
water	+	18	4	118	9	10	3	39	5
DMSO	+	14	2	101	13	9	1	31	3
DMF	+	13	-	87	-	10	-	23	-
ethanol	+	29	-	113	-	13	-	45	-
acetone	+	16	3	114	5	11	1	43	7
EGDE[2]	+	16	-	118	9	10	-	36	-
2-AA	+	154	34	1177	246	225	100	1236	195

[2]) Ethylene glycol dimethylether

VP PU 1806
Salmonella/Microsome Test
Study No. T 5053818
BAYER AG

Summary of historical negative and positive controls of experiments performed from July to December 1993 using mean values present medians (Z) and semi-Q range (QR)

Compound and S9 Mix		Strain TA 1535 Z	TA 1535 QR	TA 100 Z	TA 100 QR	TA 1537 Z	TA 1537 QR	TA 98 Z	TA 98 QR
water	-	11	2	82	21	7	3	21	2
DMSO	-	11	2	76	15	8	2	19	2
ethanol	-	17	-	121	-	8	-	27	-
acetone	-	16	-	92	-	9	-	20	-
EGDE[2]	-	17	3	95	20	9	2	18	5
Na-azid	-	643	145						
NF	-			284	74				
4-NPDA	-					65	11	58	9
30%									
water	+	16	-	127	-	6	-	26	-
DMSO	+	18	4	105	16	10	3	37	9
EGDE[2]	+	15	-	140	-	10	-	27	-
2-AA	+	220	23	448	203	88	21	478	86
10%									
water	+	12	4	100	19	8	2	27	7
DMSO	+	14	3	98	21	10	2	29	6
ethanol	+	25	-	125	-	13	-	47	-
acetone	+	15	-	113	-	10	-	32	-
EGDE[2]	+	18	-	118	11	12	1	29	3
2-AA	+	145	39	812	342	164	40	1127	223

[2]) Ethylene glycol dimethylether

Summary of historical negative and positive controls of experiments performed from January to December 1994 using mean values present medians (Z) and semi-Q range (QR)

Compound and S9 Mix		Strain TA 1535 Z	TA 1535 QR	TA 100 Z	TA 100 QR	TA 1537 Z	TA 1537 QR	TA 98 Z	TA 98 QR
water	-	10	2	89	14	8	2	22	3
DMSO	-	9	2	82	9	9	2	21	4
DMF	-	12	-	94	-	9	-	25	-
methanol	-	12	-	95	-	11	-	15	-
ethanol	-	7	2	56	10	8	2	24	11
acetone	-	7	3	79	12	6	2	18	3
EGDE[2]	-	11	3	79	10	8	2	16	6
Na-azid	-	706	98						
NF	-			263	33				
4-NPDA	-					105	35	138	23
30%									
water	+	13	-	98	-	7	-	29	-
DMSO	+	11	-	96	-	5	-	22	-
EGDE[2]	+	10	-	115	-	7	-	22	-
2-AA	+	152	24	594	140	72	29	489	93
10%									
water	+	13	2	112	15	8	2	30	7
DMSO	+	11	2	105	15	8	2	27	5
DMF	+	13	-	106	-	9	-	36	-
methanol	+	20	-	119	-	11	-	32	-
ethanol	+	10	3	84	11	4	2	28	4
acetone	+	10	-	106	11	6	3	28	2
EGDE[2]	+	14	3	114	18	8	2	29	10
2-AA	+	171	49	1111	330	103	73	1301	208

[2]) Ethylene glycol dimethylether

Summary of historical negative and positive controls of experiments performed from January to December 1995 using mean values present medians (Z) and semi-Q range (QR)

Compound and S9 Mix		Strain TA 1535 Z	QR	TA 100 Z	QR	TA 1537 Z	QR	TA 98 Z	QR
water	-	9	1	86	10	9	2	22	4
DMSO	-	9	2	81	13	8	2	20	4
ethanol	-	9	-	67	--	8	-	24	-
EGDE[2]	-	9	-	74	--	9	-	24	-
Na-azid	-	739	71						
NF	-			253	25				
4-NPDA	-					139	16	158	18
10% water	+	11	1	107	11	10	2	30	3
DMSO	+	11	2	103	16	10	3	30	5
ethanol	+	10	-	86	--	9	-	42	-
EGDE[2]	+	10	-	97	--	12	-	30	-
2-AA	+	204	48	1540	228	281	77	1461	163

[2]) Ethylene glycol dimethylether

B A Y E R A.G.
DEPARTMENT OF TOXICOLOGY
PHARMA RESEARCH CENTER
WUPPERTAL ELBERFELD
AMES TEST with : VP PU 1806

Table : 1

Study Number : T 5053818
Study Director : Dr.Herbold
Technician : Düver
Date : Oct. 7, 1996
Strain: S.typhimurium TA 1535

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	per ml 10^{*8}	QUOTIENT -S9	+S9
DMSO	7 5 7	6	1	9 7 12	9	3	122 129	12.6	1.0	1.0
16	9 10 7	9	2	5 7 9	7	2	127 140	13.4	1.4	0.8
50	6 13 11	10	4	8 12 12	11	2	122 119	12.1	1.6	1.1
158	10 7 6	8	2	8 10 5	8	3	126 138	13.2	1.2	0.8
500	8 7 9	8	1	9 13 11	11	2	137 144	14.1	1.3	1.2
1581	2 0 1	1	1	10 B 12 B 0 B	7	6	14 9	1.2**	0.2	0.8
5000	P	/	/	P	/	/	P	/	/	/
Na-azide 10	927 889 983	933	47	%	/	/	137 140	13.9	147.3*	/
2-AA 3	%	/	/	197 174 195	189	13	82 65	7.4**	/	20.2*

*: mutagenic effect
%: not tested
M: Mean
-S9: without S9 Mix
**: bacteriotoxic effect
B: Background lawn reduced
SD: Standard-Deviation
+S9: with S9 Mix
P: Precipitation

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PHARMA RESEARCH CENTER
WUPPERTAL ELBERFELD
AMES TEST with : VP PU 1806

Table : 2

Study Number : T 5053818
Study Director : Dr.Herbold
Technician : Düver
Date : Oct. 7, 1996
Strain: S.typhimurium TA 100

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{+8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	80 79 77	79		88 102 85	92	9	52 29	4.1	1.0	1.0
16	66 81 63	70	10	126 112 135	124	12	38 46	4.2	0.9	1.4
50	58 67 70	65	6	114 131 127	124	9	41 44	4.3	0.8	1.4
158	60 51 74	62	12	131 124 111	122	10	42 48	4.5	0.8	1.3
500	58 59 51	56	4	86 111 114	104	15	56 55	5.6	0.7	1.1
1581	0 0 0	0	0	2 0 3	2	2	0 0	< 0.1**	0.0	0.0
5000	P	/	/	P	/	/	P	/	/	/
NF 0.2	236 244 245	242	5	%	/	/	60 64	6.2	3.1*	/
2-AA 3	%	/	/	1290 1087 1065	1147	124	42 36	3.9**	/	12.5*

*: mutagenic effect
%: not tested
M: Mean
-S9: without S9 Mix
**: bacteriotoxic effect
P: Precipitation
SD: Standard-Deviation
+S9: with S9 Mix

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PHARMA RESEARCH CENTER
WUPPERTAL ELBERFELD
AMES TEST with : VP PU 1806

Table : 3

Study Number : T 5053818
Study Director : Dr.Herbold
Technician : Düver
Date : Oct. 7, 1996
Strain: S.typhimurium TA 1537

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{+8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	9 6 8	8	2	8 6 5	6	2	65 79	7.2	1.0	1.0
16	9 10 8	9	1	8 5 11	8	3	68 54	6.1	1.2	1.3
50	8 3 3	5	3	12 7 8	9	3	53 61	5.7	0.6	1.4
158	4 B 5 B 5 B	5	1	6 6 4	5	1	73 77	7.5	0.6	0.8
500	2 B 6 B 4 B	4	2	8 B 7 B 7 B	7	1	85 75	8.0	0.5	1.2
1581	0 0 0	0	0	0 0 0	0	0	0 0	< 0.1**	0.0	0.0
5000	P	/	/	P	/	/	P	/	/	/
4-NPDA 10	160 178 174	171	9	%	/	/	72 76	7.4	22.3*	/
2-AA 3	%	/	/	322 311 247	293	41	64 52	5.8	/	46.3*

*: mutagenic effect
%: not tested
M: Mean
-S9: without S9 Mix
**: bacteriotoxic effect
B: Background lawn reduced
SD: Standard-Deviation
+S9: with S9 Mix
P: Precipitation

B A Y E R A.G.
DEPARTMENT OF TOXICOLOGY
PHARMA RESEARCH CENTER
WUPPERTAL ELBERFELD
AMES TEST with : VP PU 1806

Table : 4

Study Number : T 5053818
Study Director : Dr.Herbold
Technician : Düver
Date : Oct. 7, 1996
Strain: S.typhimurium TA 98

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	per ml 10^{*8}	QUOTIENT -S9	+S9
DMSO	21 22 19	21	2	29 27 27	28	1	214 184	19.9	1.0	1.0
16	36 26 24	29	6	31 29 39	33	5	240 248	24.4	1.4	1.2
50	28 19 26	24	5	40 51 44	45	6	250 202	22.6	1.2	1.6
158	27 25 25	26	1	30 35 33	33	3	193 212	20.3	1.2	1.2
500	18 17 14	16	2	45 52 37	45	8	243 209	22.6	0.8	1.6
1581	0 0 0	0	0	0 0 0	0	0	74 60	6.7**	0.0	0.0
5000	P	/	/	P	/	/	P	/	/	/
4-NPDA 0.5	165 192 186	181	14	%	/	/	196 201	19.9	8.8*	/
2-AA 3	%	/	/	1890 1921 1937	1916	24	180 185	18.3	/	69.3*

*: mutagenic effect
%: not tested
M: Mean
-S9: without S9 Mix
**: bacteriotoxic effect
P: Precipitation
SD: Standard-Deviation
+S9: with S9 Mix

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DEPARTMENT OF TOXICOLOGY
PHARMA RESEARCH CENTER
WUPPERTAL ELBERFELD
AMES TEST with : VP PU 1806

Table : 5

Study Number : T 5053818
Study Director : Dr.Herbold
Technician : Düver
Date : Oct. 14, 1996
Strain: S.typhimurium TA 1535

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	per ml 10^{+8}	QUOTIENT -S9	+S9
DMSO	10 9 6	8	2	10 13 12	12	2	133 144	13.9	1.0	1.0
19	9 7 11	9	2	8 10 9	9	1	137 144	14.1	1.1	0.8
38	11 6 13	10	4	6 7 7	7	1	140 142	14.1	1.2	0.6
76	8 11 11	10	2	10 11 11	11	1	147 155	15.1	1.2	0.9
152	8 12 11	10	2	9 9 7	8	1	157 157	15.7	1.2	0.7
304	13 10 7	10	3	12 8 8	9	2	171 145	15.8	1.2	0.8
608	B	/	/	B	/	/	72 63	6.8**	/	/
Na-azide 10	872 857 784	838	47	%	/	/	142 145	14.4	100.5*	/
2-AA 3	%	/	/	277 192 237	235	43	111 91	10.1**	/	20.2*

*: mutagenic effect
%: not tested
M: Mean
-S9: without S9 Mix
**: bacteriotoxic effect
B: Background lawn reduced
SD: Standard-Deviation
+S9: with S9 Mix

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DEPARTMENT OF TOXICOLOGY
PHARMA RESEARCH CENTER
WUPPERTAL ELBERFELD
AMES TEST with : VP PU 1806

Table : 6

Study Number : T 5053818
Study Director : Dr.Herbold
Technician : Düver
Date : Oct. 14, 1996
Strain: S.typhimurium TA 100

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{+8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	80 85 86	84	3	93 93 80	89	8	99 90	9.5	1.0	1.0
19	84 101 72	86	15	112 140 138	130	16	112 115	11.4	1.0	1.5
38	81 71 77	76	5	152 178 129	153	25	118 122	12.0	0.9	1.7
76	71 88 75	78	9	141 148 134	141	7	104 119	11.2	0.9	1.6
152	79 86 93	86	7	130 148 163	147	17	128 139	13.4	1.0	1.7
304	100 84 50	78	26	138 137 136	137	1	119 157	13.8	0.9	1.5
608	39 B 24 B 25 B	29	8	48 B 39 B 39 B	42	5	120 92	10.6	0.4	0.5
NF 0.2	211 235 239	228	15	%	/	/	148 112	13.0	2.7*	/
2-AA 3	%	/	/	918 900 892	903	13	91 88	9.0	/	10.2*

*: mutagenic effect
%: not tested
M: Mean
-S9: without S9 Mix
B: Background lawn reduced
SD: Standard-Deviation
+S9: with S9 Mix

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DEPARTMENT OF TOXICOLOGY
PHARMA RESEARCH CENTER
WUPPERTAL ELBERFELD
AMES TEST with : VP PU 1806

Table : 7

Study Number : T 5053818
Study Director : Dr.Herbold
Technician : Düver
Date : Oct. 14, 1996
Strain: S.typhimurium TA 1537

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{*8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	9 9 7	8	1	7 7 5	6	1	170 169	17.0	1.0	1.0
19	5 7 6	6	1	10 10 6	9	2	163 188	17.6	0.7	1.4
38	5 8 9	7	2	9 6 9	8	2	163 190	17.7	0.9	1.3
76	7 5 7	6	1	8 6 8	7	1	210 202	20.6	0.8	1.2
152	9 11 9	10	1	6 9 6	7	2	178 183	18.1	1.2	1.1
304	5 8 7	7	2	7 8 8	8	1	212 186	19.9	0.8	1.2
608	B	/	/	6 B 7 B 0 B	4	4	173 200	18.7	/	0.7
4-NPDA 10	86 88 103	92	9	%	/	/	178 182	18.0	11.1*	/
2-AA 3	%	/	/	266 269 233	256	20	169 165	16.7	/	40.4*

*: mutagenic effect
%: not tested
M: Mean
-S9: without S9 Mix
B: Background lawn reduced
SD: Standard-Deviation
+S9: with S9 Mix

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PHARMA RESEARCH CENTER
WUPPERTAL ELBERFELD
AMES TEST with : VP PU 1806

Table : 8

Study Number : T 5053818
Study Director : Dr.Herbold
Technician : Düver
Date : Oct. 14, 1996
Strain: S.typhimurium TA 98

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{+8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	25 19 18	21	4	24 29 25	26	3	236 241	23.9	1.0	1.0
19	22 26 26	25	2	39 37 26	34	7	247 223	23.5	1.2	1.3
3	28 22 23	24	3	51 50 42	48	5	237 253	24.5	1.2	1.8
76	20 24 18	21	3	53 38 52	48	8	252 244	24.8	1.0	1.8
152	28 19 20	22	5	42 33 42	39	5	248 253	25.1	1.1	1.5
304	23 16 13	17	5	48 53 50	50	3	223 224	22.4	0.8	1.9
608	5 B 7 B 9 B	7	2	30 B 18 B 18 B	22	7	243 223	23.3	0.3	0.8
4-NPDA 0.5	163 171 186	173	12	%	/	/	262 245	25.4	8.4*	/
2-AA 3	%	/	/	1699 1614 1872	1728	131	214 217	21.6	/	66.5*

*: mutagenic effect
%: not tested
M: Mean
-S9: without S9 Mix
B: Background lawn reduced
SD: Standard-Deviation
+S9: with S9 Mix

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DEPARTMENT OF TOXICOLOGY
PHARMA RESEARCH CENTER
WUPPERTAL ELBERFELD
AMES TEST with : VP PU 1806

Table : 9

Study Number : T 5053818
Study Director : Dr.Herbold
Technician : Düver
Date : Oct. 21, 1996
Strain: S.typhimurium TA 100

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE 10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{*8}	QUOTIENT +S9
DMSO	82 107 84	91	14	93 107	10.0	1.0
75	138 125 134	132	7	134 147	14.1	1.5
150	120 136 111	122	13	86 106	9.6	1.3
300	134 153 140	142	10	94 137	11.6	1.6
450	138 138 149	142	6	102 105	10.4	1.6
600	82 B 79 B 79 B	80	2	96 100	9.8	0.9
750	77 B 82 B 55 B	71	14	15 15	1.5**	0.8
900	52 B 70 B 68 B	63	10	7 6	0.7**	0.7
2-AA 3	730 559 525	605	110	84 70	7.7	6.6*

*: mutagenic effect
+S9: with S9 Mix
M: Mean
**: bacteriotoxic effect
B: Background lawn reduced
SD: Standard-Deviation

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PHARMA RESEARCH CENTER
WUPPERTAL ELBERFELD
AMES TEST with : VP PU 1806

Table : 10

Study Number : T 5053818
Study Director : Dr.Herbold
Technician : Düver
Date : Oct. 21, 1996
Strain: S.typhimurium TA 98

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE 10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{+8}	QUOTIENT +S9
DMSO	27 30 24	27	3	340 337	33.9	1.0
75	40 40 55	45	9	324 325	32.5	1.7
150	39 35 38	37	2	333 315	32.4	1.4
300	30 37 46	38	8	306 305	30.6	1.4
450	45 38 31	38	7	310 289	30.0	1.4
600	37 31 37	35	3	327 320	32.4	1.3
750	26 22 37	28	8	286 274	28.0**	1.0
900	19 25 29	24	5	196 213	20.5**	0.9
2-AA 3	1654 1682 1425	1587	141	327 354	34.1	58.8*

+S9: with S9 Mix
*: mutagenic effect
**: bacteriotoxic effect
M: Mean
SD: Standard-Deviation

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PHARMA RESEARCH CENTER
WUPPERTAL ELBERFELD
AMES TEST with : VP PU 1806

Table : 11

Study Number : T 5053818
Study Director : Dr.Herbold
Technician : Düver
Date : Nov. 4, 1996
Strain: S.typhimurium TA 100

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE 30% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{-8}	QUOTIENT +S9
DMSO	97 119 98	105	12	9 4	0.7	1.0
75	273 303 335	304	31	13 14	1.4	2.9*
150	380 338 341	353	23	13 9	1.1	3.4*
300	274 335 264	291	38	15 15	1.5	2.8*
450	102 B 106 B 147 B	118	25	4 3	0.4	1.1
600	93 B 139 B 168 B	133	38	1 2	0.2**	1.3
750	71 B 92 B 95 B	86	13	1 1	0.1**	0.8
900	97 B 90 B 113 B	100	12	0 1	0.1**	1.0
2-AA 3	352 378 346	359	17	11 9	1.0	3.4*

*: mutagenic effect
+S9: with S9 Mix
M: Mean
**: bacteriotoxic effect
B: Background lawn reduced
SD: Standard-Deviation

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PHARMA RESEARCH CENTER
WUPPERTAL ELBERFELD
AMES TEST with : VP PU 1806

Table : 12

Study Number : T 5053818
Study Director : Dr.Herbold
Technician : Düver
Date : Nov. 4, 1996
Strain: S.typhimurium TA 98

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE 30% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{+8}	QUOTIENT +S9
DMSO	26 34 37	32	6	236 256	24.6	1.0
75	92 123 95	103	17	248 260	25.4	3.2*
150	74 62 78	71	8	258 240	24.9	2.2*
300	75 72 71	73	2	256 259	25.8	2.2*
450	38 42 38	39	2	213 269	24.1	1.2
600	58 37 46	47	11	269 250	26.0	1.5
750	26 B 35 B 36 B	32	6	106 99	10.3**	1.0
900	44 B 38 B 34 B	39	5	133 136	13.5**	1.2
2-AA 3	618 716 625	653	55	250 238	24.4	20.2*

*: mutagenic effect
+S9: with S9 Mix
M: Mean
**: bacteriotoxic effect
B: Background lawn reduced
SD: Standard-Deviation

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